insertion are exemplified in Table 10 on page 114, in particular, see the plant in range 18, row 129, stake B or the plant in range 20, row 102, stake A, both of which are free of the NPTII marker gene which was originally present in the transgene insertion.

B. Provisional Obviousness-Type Double Patenting Rejection

Pending claims 52-65 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over allowed claims of copending application Serial No. 09/521,557. The Examiner asserts that while the claims are not identical, they are not patentably distinct from each other as it would have been obvious to one of ordinary skill in the art to utilize the direct repeat-mediated method of transgene alteration in the copending application to obtain the direct repeat-mediated method of transgene deletion to obtain a marker free plant as claimed in the instant application.

The allowed claims in the copending application are directed to a method of preparing a fertile transgenic cereal plant having an altered transgene insertion while the instant claims are directed to a method of preparing a marker-free, fertile transgenic plant having all marker gene sequences removed from the transgene insert. The claims are distinct, e.g. the instant claims could be infringed by applying the method to a dicot plant without infringing the allowed claims in the copending application. The allowed claims in the copending application could be infringed by deleting one, but not every, marker gene from a transgene insert in a cereal plant without infringing the instant claim. In addition the allowed claims could be infringed by addition of a transgene without infringing the instant claims. Clearly, there is two-way distinctness. Moreover, the Examiner has provided no motivation as to why a person of ordinary skill in the art would apply a limited method to plants other than cereal plants. Rejections for obviousness-type double patenting must be support by clear evidence. See *In re Kaplan* 229 USPQ 678 (Fed. Cir. 1986). Absent such motivation applicants submit that the rejection is improper.

The Examiner further argues that the instant claims drawn to marker gene deletion in any plant are rendered obvious by the allowed claims of the copending application which are limited to cereals and encompass any type of transgene alteration. Applicants submit that amendment to claim 52, requiring removal of all marker genes, renders the instant claims even more patentably distinct from the allowed claims in the copending application which do not require or suggest

removal of all marker gene sequences. Applicant requests reconsideration and withdrawal of the provisional obviousness-type double patenting rejection.

C. **Rejection Under 35 U.S.C. §102(b)**

Pending claims 52-53 and 58 are rejected under 35 U.S.C. 102(b) being anticipated by each of Swoboda *et al.*, EMBO J, 13(2):484-489 (Swoboda *et al.)* and Assaad and Signer, *Genetics,* 132:553-566, 1992 (Assaad *et al.*).

Swoboda *et al.* discloses a transformed *Arabidopsis* plant with a hygromycin gene flanked by two sections of a GUS marker gene. The hygromycin gene is deleted by homologous recombination resulting in hygromycin sensitivity and GUS activity upon restoration of the GUS gene. Assaad *et al.* discloses a transformed *Arabidopsis* plant with a hygromycin gene flanked by two sections of an NPTII gene. The hygromycin gene is deleted by homologous recombination resulting in hygromycin sensitivity and kanamycin resistance upon restoration of the NPTII gene.

Applicants submit that amendments to claim 52 obviate this rejection as the marker-free fertile transgenic plants of the instant invention have all marker genes removed from the transgene insertion. Neither Swoboda *et al.* nor Assaad *et al.* teach or suggest the production of a marker-free transgenic plant in which all marker gene sequences are removed from the transgene insertion. More specifically, these references demonstrate the removal of one of two marker genes present in a transgenic plant, but they do not teach or suggest the complete removal of all transgene marker genes or sequences and thus are not "marker-free." In each case, the transgenic plants still comprise a marker gene in the genome.

Furthermore, neither Swoboda *et al.* nor Assaad *et al.* teach the benefit of marker-free plants. Swoboda *et al.*, merely focus upon investigating somatic and germinal rates of homologous recombination in plants. Assaad *et al.*, report the ability to demonstrate intrachromosomal homologous recombination and it's occurrence over the life cycle of a plant. While each reference points out interesting findings about *in planta* recombination, neither reference, alone or in combination, provides any motivation to a person of ordinary skill to use homologous recombination to produce a plant that is free of marker sequences or free of any transgenic sequences at all. As demonstrated in the data provided in Table 10, page 114, applicants have produced plants in which all marker sequences have been deleted, that is to say,

they are marker-free (see in particular plant in range 18, row 129, stake B or the plant in range 20, row 102, stake A, both of which lack the NPTII marker gene present in the original transgene insertion). Applicants submit that amendments to claim 52 obviate rejections over Swoboda *et al.* and Assaad *et al.* and respectfully request withdrawal of the section 102(b) rejection.

The Examiner argues further that the method of the instant invention does not exclude the use of marker gene fragments to constitute the directly repeated sequences flanking the gene to be deleted. Newly amended claim 52 obviates this rejection in that the marker-free fertile transgenic plant of the instant invention has all marker gene sequences removed from the transgene insertion; thus, the directly repeated sequences could not comprise marker genes or marker gene fragments. A marker-free plant could not be generated by the method of the instant invention should the directly repeated sequences constitute marker genes or marker gene fragments.

Applicants submit that newly amended claims 52-65 are patentable over the art of record and the copending application. An early favorable action with allowance of these claims is earnestly solicited.

Respectfully submitted,

Pamela C Ball

Pamela C. Ball
Registration Number P-53,963
Agent for Applicants
860 572 5265

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

52. (currently amended): A method of preparing a marker-free fertile transgenic plant having all marker gene sequences removed from transgene insertions comprising:

a) obtaining a first fertile transgenic plant homozygous for a transgene insertion DNA sequence, wherein the transgene insertion DNA sequence comprises at least one marker gene DNA sequence flanked by directly repeated DNA sequences, wherein said directly repeated sequences are not recognized by a site-specific recombinase enzyme;

b) obtaining a plurality of progeny of any generation of the first fertile transgenic plant; and

c) selecting a progeny fertile transgenic plant wherein ~~the marker gene is~~ all marker gene sequences are deleted.

53. (currently amended): The method of claim 52 wherein the marker gene comprises a selectable marker gene or reporter gene.

54. (original): The method of claim 53 wherein the selectable marker gene comprises a *bar*, *nptII*, or a glyphosate resistant EPSPS enzyme gene.

55. (original): The method of claim 53 wherein the reporter gene comprises a *uid*A gene, a *gfp* gene or an R-locus gene.

56. (original): The method of claim 52 wherein the plant is a monocot.

57. (original): The method of claim 56 wherein the monocot plant is a maize, barley, sorghum, wheat, rye or rice plant.

58. (original): The method of claim 52 wherein the plant is a dicot.

59. (original): The method of claim 58 wherein the dicot plant is a soybean, cotton, canola, or potato plant.

60. (original): The method of claim 52 wherein said plant is maize and the plurality of progeny plants are obtained by self pollination.

61. (original): The method of claim 52 wherein said plant is maize and the plurality of progeny plants are obtained by outcrossing to produce hybrid progeny.

62. (original): The method of claim 52 wherein said plant is maize and the plurality of progeny plants are obtained by inbreeding to produce inbred plants.

63. (original): The method of claim 52 wherein the marker gene is deleted at a frequency of at least 0.1%.

64. (original): The method of claim 52 wherein the marker gene is deleted at a frequency of at least 0.6%.

65. (original): The method of claim 52 wherein the marker gene is deleted at a frequency of at least 2%.